neutrophils similar to those observed with IL-8. On the other hand, the truncated form of PF4 (DLQ-PF4) as well as IL-8 with ELQ in place of ELR were inactive [15].

After recognition of the importance of the *N*-terminus, several analogues of the amino-terminally truncated IL-8 were synthesized as potential IL-8 receptor antagonists. Deletions or amino-acid replacements in the ELR region led to the envisaged goal. The most potent antagonists identified so far are R-IL-8 and AAR-IL-8. They inhibit IL-8 receptor binding, exocytosis (IC_{50} 0.3 μM], hemotaxis and the respiratory burst. Inhibition is restricted to responses elicited by IL-8, GROα or NAP-2, and no effect is observed when the unrelated agonists fMet-Leu-Phe or C5a are used as stimuli, demonstrating that they are selective for IL-8 receptors [16].

Since neutrophils always express high numbers of both IL-8 receptors, it was interesting to explore the function of the single species. IL-8R1 and IL-8R2 were, therefore, expressed separately in Jurkat cells. Challenge with IL-8 or GROα showed that both receptors function independently and mediate the same pattern of responses to different CXC chemokines [17].

References

1 Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines - CXC and CC chemokines. Adv Immunol 1994; 55: 97-179.

2 Baggiolini M, Dahinden CA. CC Chemokines in allergic inflammation. Immunology Today 1994; 15: 127-133.

3 Baggiolini M, Imboden P, Detmers P. Neutrophil activation and the effects of interleukin-8/neutrophil-activating peptide 1 [IL-8/NAP-1]. In: Baggiolini M. and Sorg C, eds. Cytokines. Vol. 4. Interleukin-8 [NAP-1] and related chemotactic cytokines. Basel: Karger, 1992, pp. 1-17.

4 Baggiolini M, Wymann MP. Turning on the respiratory burst. TIBS 1990; 15: 69-72.

5 Baggiolini M, Kernen P. Neutrophil activation - Control of shape change, exocytosis and the respiratory burst. News Physiol Sci 1992; 7: 215-219.

6 Thelen M, Dewald B, Baggiolini M. Neutrophil signal transduction and activation of the respiratory burst. Physiol Rev 1993; 73: 797-821.

7 Dewald B, Thelen M, Baggiolini M. Two transduction sequences are necessary for neutrophil activation by receptor agonists. J Biol Chem 1988; 263: 16,179-16,184.

8 Thelen M, Wymann MP, Langen H. Wortmannin binds specifically to 1-phosphatidylinositol 3-kinase while inhibiting guanine nucleotide-binding protein-coupled receptor signaling in neutrophil leukocytes. Proc Natl Acad Sci USA 1994; 91: 4960-4964.

9 Moser B, Schumacher C, von Tscharner V, Clark-Lewis I, Baggiolini M. Neutrophil-activating peptide 2 and *gro*/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. J Biol Chem 1991; 266: 10666-10671.

10 Schumacher C, Clark-Lewis I, Baggiolini M, Moser B. High- and low-affinity binding of GROα and neutrophil-activating peptide 2 to interleukin 8 receptors on human neutrophils. Proc Natl Acad Sci USA 1992; 89: 10542-10546.

11 Holmes WE, Lee J, Kuang W-J, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. Science 1991; 253: 1278-1280.

12 Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 1991; 253: 1280-1283.

13 Clark-Lewis I, Schumacher C, Baggiolini M, Moser B. Structure-activity relationships of interleukin-8 determined using chemically synthesized analogs. Critical role of NH_2-terminal residues and evidence for uncoupling of neutrophil chemotaxis, exocytosis, and receptor binding activities. J Biol Chem 1991; 266: 23,128-23,134.

14 Hébert CA, Vitangcol RV, Baker JB. Scanning mutagenesis of interleukin-8 identifies a cluster of residues required for receptor binding. J Biol Chem 1991; 266: 18989-18994.

15 Clark-Lewis I, Dewald B, Geiser T, Moser B, Baggiolini M. Platelet factor 4 binds to interleukin 8 receptors and activates neutrophils when its N terminus is modified with Glu-Leu-Arg. Proc Natl Acad Sci USA 1993; 90: 3574-3577.

16 Moser B, Dewald B, Barella L, Schumacher C, Baggiolini M, Clark-Lewis I. Interleukin-8 antagonists generated by *N*-terminal modification. J Biol Chem 1993; 268: 7125-7128.

17 Loetscher P, Seitz M, Clark-Lewis I, Baggiolini M, Moser B Both interleukin-8 receptors independently mediate chemotaxis. Jurkat cells transfected with IL-8R1 or IL-8R2 migrate in response to IL-8, GROα and NAP-2. FEBS Lett. 1994; 341: 187-192.

Granules and secretory vesicles of the human neutrophil

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Introduction

In order to fulfill its role in host defence against invading microorganisms, the human neutrophil is equipped with a battery of proteases and bactericidal substances and has the ability to assemble an electron transport chain (the NADPH oxidase) capable of generating huge amounts of reactive oxygen species, all contributing to the bactericidal and tissue destructive armory of this cell. As a mobile phagocyte, the neutrophil must circulate throughout the entire vascular bed without compromising the integrity of the microcirculation, yet, at sites of incipient inflammation, the cell must be able to adhere rapidly to endothelium, diapedese and migrate into tissues to phagocytose and kill offending microorganisms. Thus, the neutrophil must be able to adjust rapidly to changes in its surroundings in order to meet the requirements necessary for optimal function in the inflammatory reaction.

The neutrophil is equipped with a plethora of discrete granule subsets and vesicles each with their characteristic protein profile (Table 1). The strictly hierarchic mobilization of these [1,2], offers a structural basis for orchestrating the step-wise metamorphosis of the neutrophil from a stage of circulating quiescence to a stage of maximal metabolic and destructive activity, characteristic of neutrophils in the inflammatory focus.

Secretory vesicles

Secretory vesicles have only recently been described [3,4]. These are small vesicles scattered throughout the cytoplasm that are formed during late stages of neutrophil development in the bone marrow [5]. Secretory vesicles are endocytic in origin and contain plasma proteins that are exocytosed when the membrane of secretory vesicles fuses with the plasma membrane [6]. Despite their endocytic origin, the mobilization of secretory vesicles, like that of granules, is controlled.

Secretory vesicles are uniquely sensitive to mobilization and are efficiently mobilized by stimulation of neutrophils with a host of inflammatory mediators that barely mobilize granules [1,7]. These include nanomolar concentrations of fMLP, LTB_4, PAF, TNF, IL-8 andGM-CSF [7]. The application of high voltage free flow electrophoresis for separation of secretory vesicles and plasma membranes [8] has permitted further characterization of secretory vesicles [9–11] beyond the original description as a readily mobilizable reservoir of alkaline phosphatase [9]. Secretory vesicles are the main reservoir of CR1 (CD35) [12], Fcγ-RIII (CD16) [13], and most likely also of neutral endopeptidase CD10[14], and Decay Accelerating Factor (DAF)[15]. In addition, secretory vesicles have certain membrane proteins in common with the peroxidase-negative subset of neutrophil granules. These include the receptor for formylated chemotactic peptides [16], urokinase type plasminogen activator [16], Mac-1 (CD11b/CD18) [19] and cytochrome b_{558} [17] (Table 1). The significance of secretory vesicles is thus attributed to their function as a readily mobilizable reservoir of functional proteins that can be incorporated into the plasma membrane without the concomitant liberation of potential hazardous granule content.

The initial contact of neutrophils with endothelium is mediated through L-selectin [18,19] and PSGL-1[20], glycoproteins located on the tips of microvilli [7] capable of binding to sialyl Lewis(x)-containing structures including P-selectin on activated endothelium. Signals generated through L-selectin [17] and probably also exposure to inflammatory mediators presented by the endothelium [22] result in mobilization of secretory vesicles, thus changing the neutrophil from a rolling, L-selectin presenting cell, to a Mac-1 presenting cell, capable

Table 1. Content of human neutrophil granules and secretory vesicles

Azurophil granules	**Specific granules**	**Gelatinase granules**	**Secretory vesicles**
Membrane:	*Membrane:*	*Membrane:*	Membrane:
CD63	CD15 antigens,	Cytochrome b_{558},	Alkaline phosphatase,
CD68	CD66,	Diacylglycerol-	CR1 (CD35),
	CD67,	deacylating enzyme,	Cytochrome b_{558},
Matrix:	Cytochrome-b_{558},	FMLP-R,	FMLP-R,
	FMLP-R,	Mac-1 (CD11b/CD18),	Mac-1 (CD11b/CD18),
Acid β-glycerophosphatase,	Fibronectin-R,		Uroplasminogen activator-R,
Acid mucopolysaccharide,	G-protein $_{\alpha}$-subunit,	*Matrix:*	CD10, CD13, CD45*
α_1-antitrypsin,	Laminin-R,		FcγRIII (CD16),
α-mannosidase,	Mac-1 (CD11b/CD18),	Acetyltransferase,	C1q-receptor,
Azurocidin/CAP37/	NB 1 antigen,	β_2-microglobulin,	DAF*
Heparin binding protein,	19 kD-protein,	Gelatinase,	
Bactericidal permeability	155 kD-protein,	Lysozyme	*Matrix:*
Increasing protein,	Rap1, Rap2		
β-glycerophos-phatase,	Thrombospondin-R,		Plasma proteins (incl. tetranectin)
β-glucuronidase,	TNF-Receptor,		
Cathepsins,	Vitronectin-R		
Defensins,			
Elastase,	*Matrix:*		
Lysozyme,			
Myeloperoxidase,	β_2-microglobulin,		
N-Acetyl-β-glucosaminidase,	Collagenase,		
Proteinase-3,	Gelatinase,		
Sialidase	Histaminase,		
	Heparanase,		
	Lactoferrin,		
	Lysozyme,		
	NGAL,		
	Plasminogen activator,		
	Sialidase,		
	Vit. B_{12}-binding protein		

* Means that this localization is probably based on kinetics of upregulation in response to stimulation with inflammatory mediators, but has not yet been demonstrated by subcellular localization or immunocytochemistry. References to this table are given in [29].

of firm adhesion to ICAM-1 present on endothelium. Secretory vesicles are therefore most likely of primary importance in determining the interaction of circulating neutrophils with endothelium. In accordance, secretory vesicles are totally mobilized in cells that have diapedesed into a skin window chamber[12].

Granules

It has long been known that neutrophils contain two main types of granules, the peroxidase-positive granules also known as azurophil granules or primary granules, and the peroxidase-negative granules, previously also called specific granules[23].

Subcellular fractionation experiments in combination with double-labelling immunoelectron microscopy have shown that peroxidase-negative granules exist as a heterogenous continuum from granules high in lactoferrin but devoid of gelatinase through granules containing both gelatinase and lactoferrin to granules low in lactoferrin and high in gelatinase [24]. These latter granules are referred to as gelatinase granules [25], whereas granules that contain lactoferrin are referred to as specific granules, whether or not these are lactoferrin-only granules or double-labelled granules, positive for both lactoferrin and gelatinase. This heterogeneity, which most likely is a result of controlled timing of biosynthesis of granule proteins [26], is not only structural but also functional, since gelatinase granules are always mobilized more extensively than double-labelled granules which again are mobilized more readily than lactoferrin-only granules [1,2]. Stimulation of neutrophils in suspension with nanomolar concentrations of fMLP may mobilize as much as 25% of total cell gelatinase but only 2-5% of the total amount of lactoferrin [2,27].

In contrast to the heterogeneity in size and matrix content of these granule subsets, their membrane seems to be quite similar, sharing Mac-1, cytochrome b, fMLP receptors, and receptors for urokinase type plasminogen activator [16,25]. Thus, this structural and functional heterogeneity seems to be more relevant in terms of differential release of matrix proteins rather than upregulation of membrane proteins. The preferential mobilization of gelatinase granules may be of primary importance during diapedesis of neutrophils since type IV collagen, a preferred substrate for gelatinase [28], is the main collagen constituent of basement membranes. Collagenase which degrades the interstitial type I-III collagens is located in specific granules that are mobilized after gelatinase granules.

Arriving at sites of inflammation, as observed in a skin window chamber [12], neutrophils have exocytosed 38% of their gelatinase and 21% of their lactoferrin, and only 7% of their myeloperoxidase, a constituent of peroxidase positive granules. Thus, when the neutrophil arrives to meet bacteria, their receptors for phagocytosis and NADPH oxidase components are presented at their surface, but most of the azurophil granules which contain bactericidal and proteolytic enzymes are still retained, ready for fusion with the phagocytic vacuole during ingestion of the microorganisms.

Epilogue

The existence of distinct and differentially mobilized intracellular granules and vesicles endows the neutrophil with the ability to time and optimize its activities according to the requirements that are imposed on it during its journey from the blood stream to the inflammatory focus. The intracellular signal-transduction pathways that control the differential exocytosis of the human neutrophil granules and secretory vesicles are largely unknown. It is known that the differential exocytosis of secretory vesicles and granules is reflected in the levels of intracellular Ca^{2+} that are needed to elicit exocytosis of each of the subpopulations of mobilizable organelles [1], but the sensors that Ca^{2+} must operate on, directly or indirectly to result in differential exocytosis, remain to be identified.

References

1 Sengeløv H, Kjeldsen L, Borregaard N. Control of exocytosis in early neutrophil activation. J Immunol 1993; 150: 1535–1543.

2 Kjeldsen L, Bainton DF, Sengeløv H, Borregaard N. Identification of NGAL as a novel matrix protein of specific granules in human neutrophils. Blood 1994; 83: 799–807.

3 Borregaard N, Miller L, Springer TA. Chemoattractant-regulated mobilization of a novel intracellular compartment in human neutrophils. Science 1987; 237: 1204–1206.

4 Borregaard N, Christensen L, Bjerrum OW, Birgens HS, Clemmensen I. Identification of a highly mobilizable subset of human neutrophil intracellular vesicles that contains tetranectin and latent alkaline phosphatase. J Clin Invest 1990; 85: 408–416.

5 Borreggard N, Lillike K, Kjeldsen L *et al.* Human neutrophil granules and secretory vesicles. Eur J Haematol 1993; 51: 187–198.

6 Borreggard N, Kjeldsen L, Rygaard K . Stimulus-dependent secretion of plasma proteins from human neutrophils. J Clin Invest 1992; 90: 86–96.

7 Borregaard N, Kjeldsen L, Sengeløv H *et al.* Simulation-dependent localization of L-selectin and Mac-1 in human neutrohpils. J Leuk Biol 1994; 56: 80–87.

8 Sengeløv H, Neilsen MH, Borregaard N. Separation of human neutrophil plasma membrane from intracellular vesicles containing alkaline phosphotase and NADPH oxidase activity by free flow electrophoresis. J Biol CHem 1992; 267: 14,912–14,917.

9 Sengeløv H, Kjeldsen L. Diamond MS, Springer TA, Borregaard N. Subcellular localization and dynamics of Mac-1 ($\alpha_m\beta_2$) in human neutrophils. J Clin Invest 1993; 92: 1467–1476.

10 Sengeløv H, Boulay F, Kjeldsen L, Borregaard N. Subcellular localization and translocation of the receptor for N-formyl-methionyl-leucyl-phenylalanine in human neutrophils. Biochem J 1994; 299: 473–479.

11 Sengeløv H, Kjeldsen L, Kroeze W, Berger W, Borregaard N. Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neturophils. J Immunol 1994; 153: 804–810.

12 Sengeløv H, Follin O, Kjeldsen L, Lollike K, Dahlgren C, Borregaard N. Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J Immunol 1995; in press.

13 Dehaas M, Kerst JM, Vanderschoot CE *et al.* Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neturophils. Blood 1994; 84: 388–3894.

14 Werfel T, Sonntag G, Weber M, Götze O. Rapid increases in the membrane expression of neutral endopeptidase (CD10), aminopeptidase N (CD13), tyrosine phsophatase (CD45), and FcτRIII (CD16) upon stimulation of human peripheral leukocytes with human C5a. J Immunol 1991; 147: 3909–3914.

15 Berger M, Medof E. Increased expression of complement decay-accelerating factor during activation of human neutrophils. J Clin Invest 1987; 79: 214–220.

16 Plesner T, Ploug M, Ellis V *et al.* the receptor for urokinase-type plasminogen activator and urokinase is translocated from two istinct intracellular compartments to the plasma membrane on stimulation of human neutrophils. Blood 1994; 83: 808-815.

17 Bjerrum OW, Borregaard N. Dual granule localization of the dormant NADPH oxidase and cytochrome b_{559} in human neutrophils. Eur J Haematol 1989; 43: 67–77.
18 von Andrian UH, Chanbers JD, Berg EL *et al.* L-selectin mediates neutrophil rolling in inflamed venules through sialyl Lewis*-dependent and -independent recognition pathways. Blood 1993; 82: 182–191.
19 Pizcueta P, Luscinskas FW. Monoclonoal antibody blockade of L-selectin inhibits mononuclear leukocyte recruitment to inflammatory sites in vivo. Am J Pathol 1994; 145: 461–469.
20 Norgard KE, Moore KL, Diaz S *et al.* Characterization of a specific ligand for P-selectin and myeloid cells. A minor glycoprotein with sialylated O-linked oligosaccharides. J Biol Chem 1993; 268: 12764-12774.
21 Laudanna C, Constantin G, Baron P *et al.* Sulfatides trigger increase of cytosolic free calcium and enhanced expression of tumor necrosis factor-alpha and interleukin-8 mRNA in human neutrophils - evidence for a role of L-selectin as a signalling molecule. J Biol Chem 1994; 269: 4021–4026.
22 Lorant DE, Patel KD, McIntyre TM, McEver RP, Precott SM, Zimmerman GA. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol 1991; 115: 223–234.
23 Baintin DF. Neutrophil granules. Br J Haematol 1975; 29: 17–22.
24 Kjeldsen L, Bainton DF, Sengeløv H, Borregaard N. Structural and functional heterogeneity among peroxidase-negative granules in human neutrophils: identification of a distinct gelatinase containing granule subset by combined immunocytochemistry and subcellular fractionation. Blood 1993; 82: 3183–3191.
25 Kjeldsen L, Sengeløv H, Lollike K, Nielsen MH, Borregaard N. Isolation and characterization of gelatinase granules from human neutrophils. Blood 1994; 83: 1640–1649.
26 Borregaard N, Sehested M, Neilsen BS, Sengeløv H, Kjeldsen L. Biosynthesis of granule proteins in normal human bone marrow cells. Gelatinase is a marker of terminal neutrophil differentiation. Blood 1995; in press.
27 Dewald B, Bretz U, Baggiolini M. Release of gelatinase from a novel secretory compartment of human neutrophils. J Clin Invest 1982; 70: 518–525.
28 Murphy G, Reynolds JJ, Bretz U, Baggiolini M. Partial purification of collenase and gelatinase from human polymorphonuclear leucocytes. Biochem J 1982; 203: 209–221.
29 Borregaard N, Kjeldsen L, Lollike K, Sengeløv H. Granules and secretory vesicles of the human neutrophil. IJPHO 1995; in press.

Th1 and Th2 cells in autoimmunity

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Introduction

It is now clear that CD4+ helper T cells (Th) include at least two different subsets called Th1 and Th2. These subsets have been characterized in mice [1], rats [2] and humans [3]. They have different functions and produce a different pattern of cytokines. Their role in various experimental autoimmune diseases is now well established, and increasing evidence highlight their importance in human autoimmune diseases too. However it is important to underline first that this dichotomy between Th1 and Th2 cells has been described in polarized situations and, second, that different types of regulation by T cells have been described.

The Th1 Th2 dichotomy

When a CD4+ T cell is appropriately stimulated, it will differentiate into either Th1 or Th2 type. Several factors are important in driving this response. The kind of antigen-presenting cell (APC) seems important, with macrophages and dendritic cells favouring a Th1 response and B cells a Th2 one. The strength of the interaction between the TCR and peptide is also important. A strong interaction will induce the development of Th1 cells and a weak interaction that of Th2 cells. The cytokines that are present in the environment of the CD4+ T cell at the time it encounters the peptide are also crucial. IL-12, produced by activated macrophages, and IFNγ, produced by natural killer cells under the influence of IL-12 and TNFα, will drive the differentiation towards the Th1 pathway provided that IL-4 is absent [4]. By contrast IL-4 is the cytokine that plays a key role in the differentiation of the precursor CD4+ T cell towards a Th2 phenotype [5]. The cells that produce IL-4 at this stage arepoorly characterized — they could be mast cells and a subpopulation of T cells. It is important to stress that the crucial cytokines are produced by cells involved in natural immunity, before the induction of the antigen-specific immune response.

Th1 and Th2 clones have been obtained in mice and humans. These T cell subsets produce different cytokines [6]. Th1 cells produce mainly IL-2 and IFN-γ while Th2 cells produce IL-4, 5, 6, 10 and 13. Th1 cells provide help to CD8+ T cells in the form of IL-2 which is the growth factor for both CD4+ and CD8+ T cells. Th1 cells also collaborate with macrophages since, through the IFNg they produce, they are able to increase their bactericidal properties. Finally Th1 cells help B cells to produce immunoglobulins such as IgG2a in mice, an immunoglobulin which is responsible for antibody-dependent-cell cytotoxicity. Th1 cells are therefore mainly involved in cell-mediated immunity. By contrast, Th2 cells help B cells to produce IgM, IgG1, IgA and mainly IgE which is a strictly Th2-dependent isotype. Interleukin-5 is responsible for attraction and activation of eosinophils. Th2 cells are therefore implied in humoral immunity [6]. These T cell subsets have a regulatory role, with IFNγ produced by Th1 cells down-modulating Th2 cells, and IL-10 produced by Th2 cells inhibiting Th1 cells [6].

It appears however that the situation is more complex for several reasons. i) There are CD4+ T cells, now called Th0 cells which produce both IL-4 and IFNγ; Th0 cells may represent either an autonomous or a transitory cell type [7]. ii) Several Th1 and Th2 cytokines, as defined above, are produced by cells other than T cells and, in addition, IL-10 that was initially considered as a Th2 cytokine may also be produced, at least in humans, by Th1 cells [8]. iii) The dichotomy described for CD4+ T cells also exists for CD8+ T cells, with the classical cytotoxic CD8+ T cells producing IFNγ and other non-cytotoxic CD8+ T cells producing IL-4 [9]. iiii) Finally there are CD4+ and CD8+ T cells that have a profound regulatory influence due to the TGFβ they produce in addition to other cytokines. It is interesting to note that these T cells are induced, for example, following mucosal immunization [10].